90. (new) A population of morphologically intact viable cryopreserved oocytes prepared by the method of claim 67.

REMARKS

Claims 46-65 are canceled. Claims 1-45 have been previously canceled.

REJECTION UNDER 35 USC §102

Claims 46, 48-51, 54, 55, 58 and 64-66 stand rejected under 35 USC §102(b) as anticipated by Martino, *et al.* The reference is asserted to describe a method that is in generally the same as that claimed by applicants, particularly in suspending oocytes in an equilibration medium, rinsing in a vitrification solution and dropping microdroplets of the oocyte suspension onto a solid surface held between –150° and –180° C. The solution compositions were further stated to be functionally the same, including the use of ethylene glycol as cryoprotectant, sucrose, and serum to provide surfactant properties.

Applicants have amended the claims, particularly to more clearly distinguish the compositions, by setting forth some of the differences between the claimed method and those described in the Martino, *et al.* publication. Applicants submit that the differences, while individually appearing to be small, are such that results are more than a mere optimization of the media and procedures employed by Martino, *et al.* There are, for example, four components in the rinsing solution, which are not employed by Martino, *et al.* Certainly Applicants have also used ultra rapid cooling, but Applicants believe that the differences in the compositions in combination with the series of equilibration and vitrification steps results in higher cleavages, blastocyst formation and increased number of hatched blastocysts than were obtained by Martino, *et al.*

REJECTION UNDER 35 USC §103

Claims 46-51, 54-62 and 64-66 stand rejected under 35 U.S.C. §103(a) as unpatentable under Martino, *et al.* in view of Papis, *et al.* In addition to the conditions for vitrification described by Martino, *et al.,* Papis, *et al.* is asserted to further detail the vitrification conditions, particularly in use of a 12-15 min equilibration at near physiological temperatures and use of rapid cooling by dropping oocyte microdroplet solutions into liquid nitrogen. Both cited references are further stated to have described successful cryopreservation such that thawed oocytes can be fertilized.

Applicants acknowledge that Martino, *et al.* and Papis, *et al.* were able to freeze, thaw and then demonstrate some degree of cleavage, blastocyst formation and hatching. Notwithstanding, Applicants' procedures are different and, importantly, are believed to provide improved results that are more than mere optimization of the methods described in the combination of references.

Even with the further combination of Arav, *et al.*, Saha, *et al.,* and Liu, *et al.*, claims 46-66 are not obvious. Arav, *et al.* is cited as specifically mentioning trehalose as a cryoprotectant while Saha, *et al.* teach a mixture of ethylene glycol, trehalose and polyvinylpyrrolidine. Liu, *et al.* is used to show that culture of fertilized oocytes in KSOM with cumulus cells is known in the art.

Applicants do not believe Liu, *et al.* is an applicable publication because it describes improvements to the culturing conditions for embryo development, not for vitrification methods that preserve viability. The publication also described a culturing medium for embryo development that is supplemented with free radical scavengers, thereby suggesting addition of taurine or SOD to the embryo culture. Accordingly, this publication is not properly combined with the primary reference or the other cited references.

Arav, *et al.* indicate that trehalose may be superior to sucrose for use in vitrification media; however, the recommended medium does not contain a macromolecule such as PVP and clearly teaches that propylene glycol, not ethylene glycol, should be used as the cryoprotectant. In this respect, Arav, *et al.* teaches away from using ethylene glycol and therefore is not properly combinable with the primary reference or the other references.

Saha, *et al.,* on the other hand, uses the combination of ethylene glycol, trehalose and PVP to freeze embryos, not oocytes. Applicants submit that this disclosure would not suggest use of this solution for vitrification of oocytes. Because this reference does not apply to oocyte vitrification, Applicants' believe it is not properly combinable with the primary reference or the other references.

Thus, further combining Arav, *et al.*, Saha, *et al.* and Liu, *et al.* with Martino, *et al.* and Papis, *et al.* provides no guidance whatsoever to further modify the solutions described in either Martino, *et al.*, in Papis, *et al.*, or in combination with those two references. Applicants do not have a free radical scavenger in their composition (Liu, *et al.*), do not freeze embryos (Saha, et al.) and use a mixture different from what is possibly suggested by combining both the Martino, *et al.* and Papis, *et al.* references.

Applicants have amended the claims to more clearly highlight the distinction in their methods that lead to the improved results.

Applicants' Improved results for survival, cleavage, blastocyst formation and hatching Comparison with data reported by Martino, *et al.*

Referring to Table 3 on page 32 of the present application, vitrified/thawed oocyte survival following in vitro fertilization was 85% compared to 98% for controls; Martino, *et al.* reported 40% vitrified/thawed oocyte survival compared with 71% for controls. Thus, compared with controls, Applicants thawed oocytes were 87% of controls while Martino, *et al.*'s were 56% of controls.

A significant improvement appears with the blastocysts. Applicants' blastocyst formation is 19% for the vitrified/thawed oocytes compared with 33% for controls at day 8. Martino, *et al.* report 15% blastocyst formation for vitrified/thawed oocytes compared with 41% for controls. Thus Applicants show a blastocyst formation of 57% compared to non-vitrified controls while Martino, *et al.* show a blastocyst formation of 37% compared with non-vitrified controls at day 8.

The results are even more distinguished for hatched blastocysts. Applicants report 12% hatched blastocysts for vitrified/thawed oocytes compared with 19% for controls. Martino, *et al.* report 4-5% hatched blastocysts for vitrified/thawed oocytes compared with 20% for controls. Thus the hatch rate for applicants is 63% compared to controls while Martino, *et al.* is 25% compared with controls. These differences are even more significant than the comparisons with initial oocyte survival rates.

Comparison with data reported by Papis, *et al.*

Applicants also looked at the data in the Papis, *et al.* publication. Table 1 of Papis, *et al.* indicates that for the best conditions, using 3% ethylene glycol in an equilibration solution, the percent of embryos developed to the hatched stage was 15%, compared to 21% of controls, representing a 71% hatch rate for the blastocysts compared with controls. According to Papis, *et al.* , the cleavage and blastocyst percents were "not significantly different from controls" although when 3% ethylene glycol was employed for equilibration, Table 1 indicates that a 30% blastocyst rate was obtained compared to 42% for controls (71% by comparison with controls) and 15% hatch rate compared with 21% for controls (again, a 71% by comparison with controls). It is not clear how long the embryos were allowed to develop before the data were collected; however, culturing was indicated to take place "up to day 10."

Applicants have used the procedures disclosed to compare fusion, cleavage, blastocyst and hatched blastocyst rates for *in vitro* development of embryos. While it is not clear that the results in Table 4 on page 23 can be directly compared with the results in Table 1 of Papis, *et al.* because the Papis, *et al.* conditions are not detailed, Applicants believe that their surprisingly high percentages of cleaved, blastocysts and hatched blastocysts compared with non-vitrified controls (94%, 93% and 90% respectively) indicate that Applicants have developed an effective and improved method for oocyte vitrification. The data show that at all stages embryo development is at least 20% better than the development percentages reported by Papis, *et al.*

Applicants employ equilibration and vitrification solutions that differ from those used by Papis, *et al.* It is evident that the Papis, *et al.* procedure is different, notably in that applicants use a higher concentration of ethylene glycol for the vitrification medium (6.28 M compared with 5.5 M used by Papis, *et al.*,) use a lower sugar concentration and use smaller microdroplets (1-2 µl vs. 6 µl) than Papis, *et al.*, to note some of the differences.

Papis, *et al.* teach that use of 4% ethylene glycol for pre-equilibration is "less efficient". Applicants, among other differences in procedure, use 4% ethylene glycol for equilibration, and as shown in Table 4, achieve better results than do Papis, *et al.* with their recommended optimum use of 3% ethylene glycol. Thus, Papis, *et al.* "teach away" by indicating that less efficient results are obtained with other than use of 3% ethylene glycol.

In this respect, even if Papis, *et al.* were combined with Martino, *et al.* or with any of the other references, the skilled artisan would not use Applicants' compositions for vitrification and would obtain inferior results.

In summary, Applicants submit that the references in any combination do not provide teaching or suggestion to use the compositions and steps of the method of the described invention. There was, and is, motivation to improve cryopreservation of oocytes, but there is no suggestion in any of the references to pick and choose from each reference to provide the compositions used by Applicants or to use the particular steps under the particular conditions described by Applicants for improved cryopreservation of oocytes.

New Claims

Claims 67-88 have been added to replace previously pending claims 46-66. Applicants have included more technical detail in the independent claim in order to provide more clarity for the new method. The technical details are supported in the application, particularly at page 12, line 17, bridging over to page 13, line 5. No new matter has been added.

CONCLUSION

Applicants believe that a complete response has been submitted. It is respectfully submitted that this application is now in condition for allowance. Should any issues remain or should the Examiner believe that a telephone conference with Applicants' attorney would be helpful in expediting prosecution, the Examiner is invited to contact the undersigned at 203.353.6848.

Respectfully submitted,

Barbara S. Kitchell

Barbara S. Kitchell, Registration No. 33,928
Edwards & Angell LLP
P.O. Box 55874
Boston, MA 02205
(203) 353-6848
(203) 975-7180

Customer No. 21874